Small animal models of filoviral hemorrhagic fever

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This talk will cover:

1. History of development of rodent models
2. Features of disease in guinea pigs and mice
3. Comparison of pathogenetic mechanisms and cause of death in rodents and primates
4. Effect of inherent resistance to infection on drug and vaccine testing

Filovirus host range

- All species/strains of Ebola and Marburg virus cause severe disease in all types of nonhuman primates so far tested.
- Filoviruses from human cases may cause *mild* illness in guinea pigs, but "adaptation" is required to produce severe disease.
- Mice over the age of about 1 week are solidly resistant to filovirus infection.
- Limited experiments have shown that Ebola Zaire virus can cause asymptomatic infection in some bat species.

Filovirus infection of guinea pigs

During the 1967 Marburg outbreak, researchers found that guinea pigs injected with virus from patients became mildly ill.

Four sequential passages of liver homogenate resulted in development of lethal disease.

The same strategy has been used to adapt Ebola Zaire and Sudan viruses to guinea pigs.

Animals become ill in 3-4 days and die in 7-10.

Filovirus infection of mice

Marburg virus caused disease in newborn mice, but not in older animals (not pursued).

Studies in 1990s found Ebola Zaire virus caused:

- Newborn mice: fatal disease
- Older suckling and adult mice: no disease
- SCID mice: slowly progressive illness with death in 3-4 weeks
- IFN-α/β receptor KO mice: onset of illness in 3-4 days and death in 5-7 days.

Findings indicate that type I IFN plays central role in resistance.

Adaptation of Ebola virus to mice

A stock of Ebola Zaire virus from the 1976 outbreak ("Mayinga") was passaged sequentially in suckling mice, beginning with newborns.

By 8th passage, virus was lethal for 15-day-old mice, but only by the intraperitoneal route.

Increased virulence was accompanied by change to a clear-plaque phenotype.

The 9th-passage virus was plaque-purified, amplified and used in subsequent experiments.

This "mouse-adapted" virus is now in use at USAMRIID, CDC, Winnipeg and Galveston.

A novel adaptation strategy

Warfield and colleagues at USAMRIID recently succeeded in adapting 4 different strains of Marburg virus to immunocompetent mice by:

1. Serially passaging virus in SCID mice until the mean time to death was markedly reduced;
2. Passaging the SCID-adapted virus in normal adult BALB/c mice until it was lethal in about one week;
3. Isolating a plaque-purified virus that retains virulence.

Features of disease in GPs and mice: routes of infection

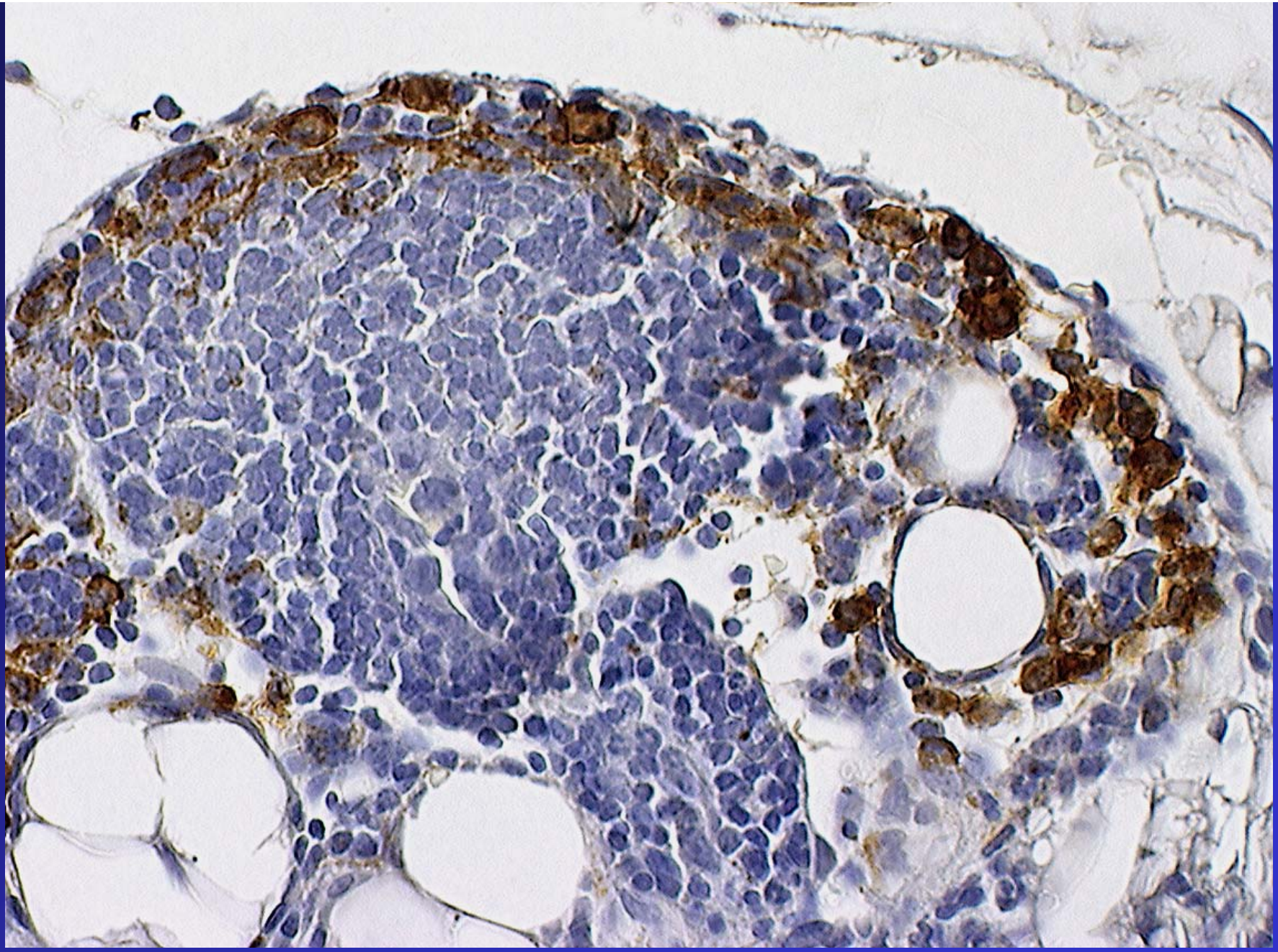
1. Guinea pigs can be infected by any route.
2. Normal immunocomptent mice are only susceptible to mouse-adapted Ebola virus injected intraperitoneally.
3. Subcutaneous injection in mice elicits a strong type I IFN response.

Features of disease in GPs and mice: kinetics of viral replication

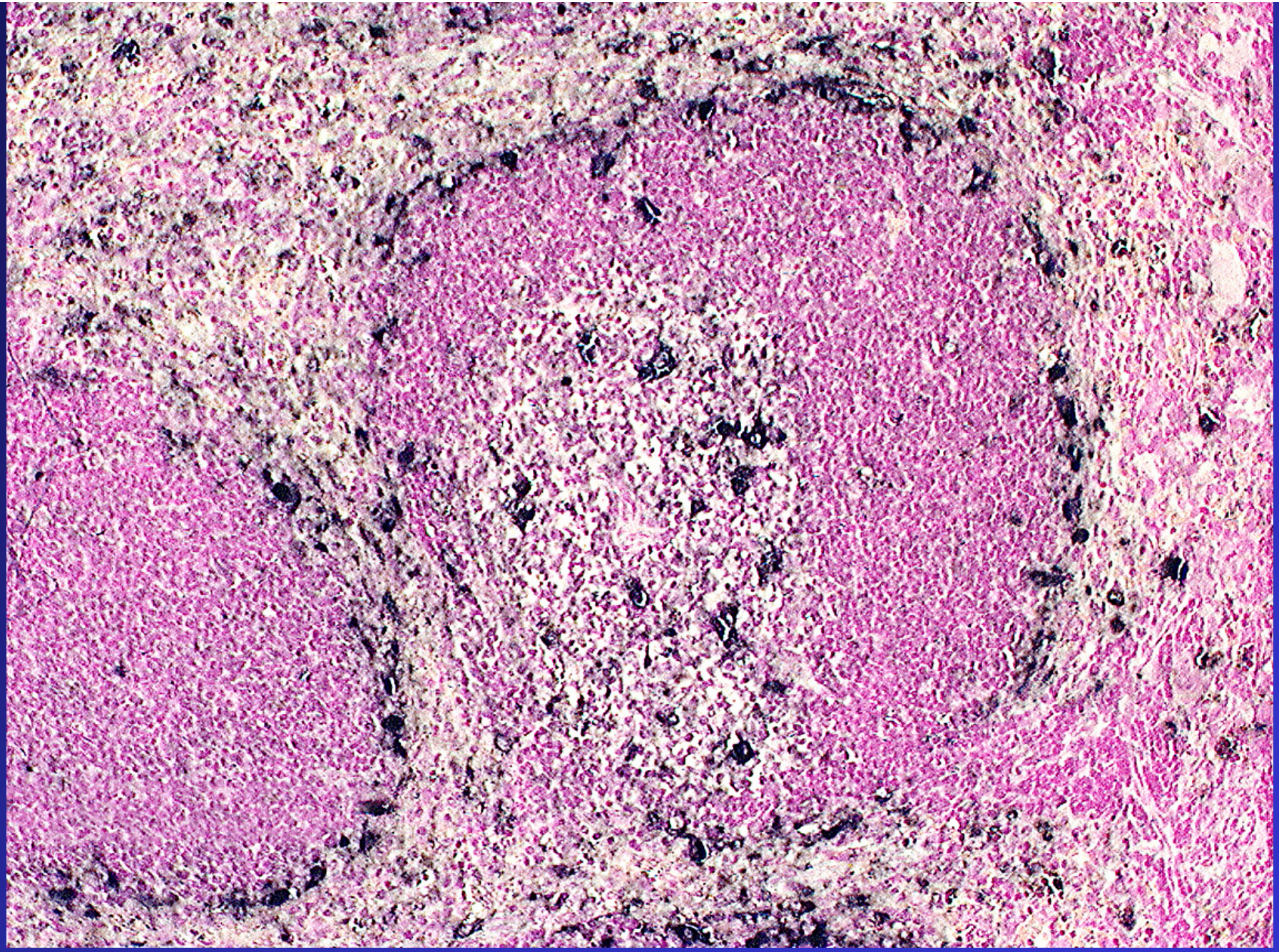
1. Virus becomes detectable in blood at day 2-3
2. Rises rapidly to range of 10^7-10^8 pfu/mL by day 4-5
3. Remains elevated through death.

Features of disease in GPs and mice: histopathology

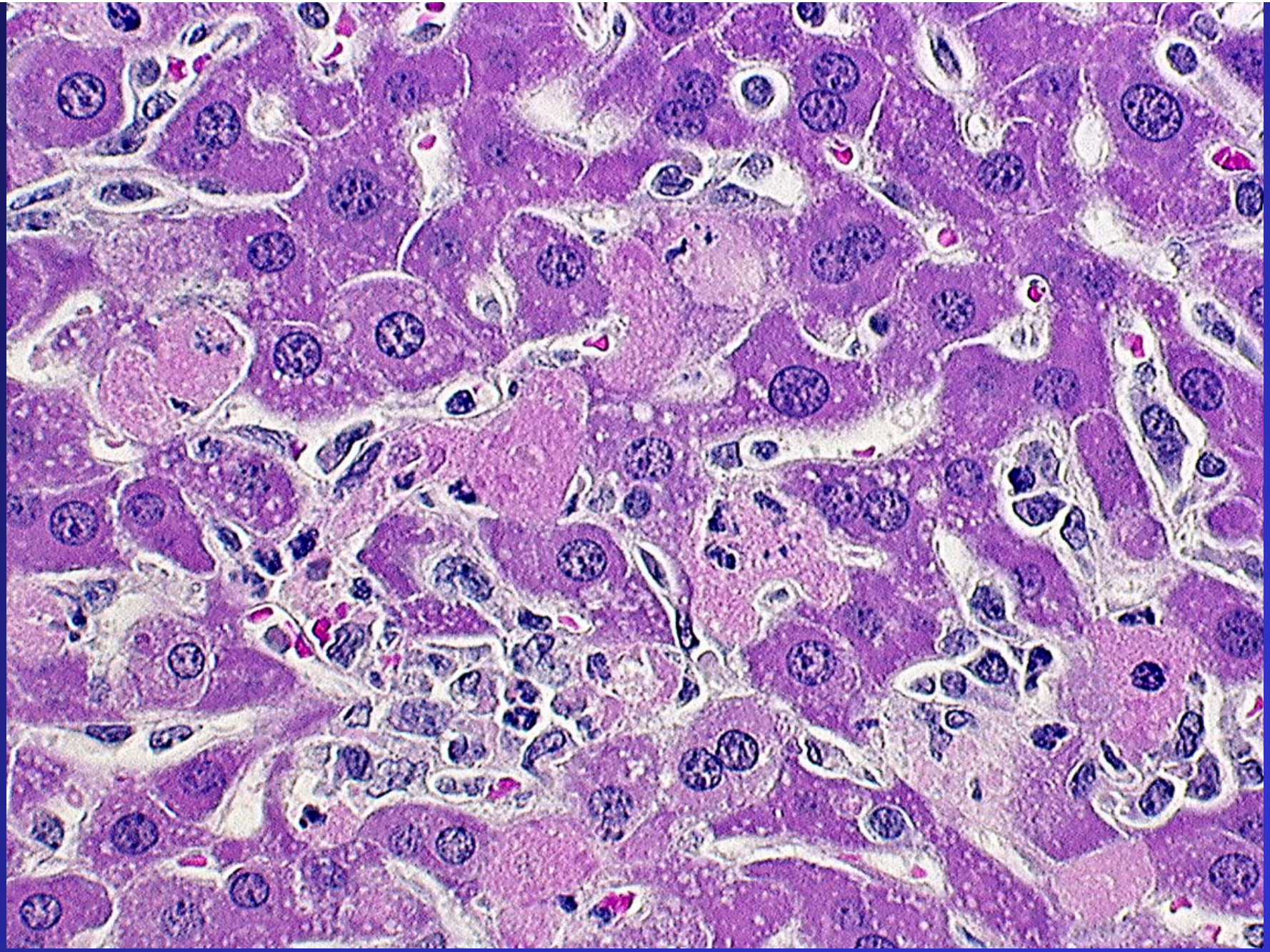
1. Macrophages are the primary site of viral replication; dendritic cells are also infected.
2. Virus extensively infects hepatocytes and parenchymal cells of some other organs.
3. Infected cells undergo necrosis.
4. Death of lymphocytes is prominent; in mice has been shown to be apoptotic.
5. Fibrin deposition is scant or absent.



Lymph node, day 2: immunohistochemistry



Spleen, day 4: in situ hybridization



Liver: hematoxylin & eosin, day 3

Features of disease in GPs and mice: changes in blood cell counts

1. An early rise in total WBCs reflects mobilization of immature granulocytes
2. Lymphocytes tend to decline over course of illness
3. Progressive thrombocytopenia seen in both species

Features of disease in GPs and mice: changes in blood chemistry

1. Liver enzymes (AST, ALT) rise markedly over course of illness.
2. LDH also elevated.
3. Signs of hemoconcentration: increased BUN, total protein, hemoglobin concentration.

Features of disease in GPs and mice: coagulation testing

1. Limited testing in normal mice infected with mouse-adapted Ebola virus showed no consistent abnormality of PT or PTT.
2. Similar tests in guinea pigs showed prolongation of both.

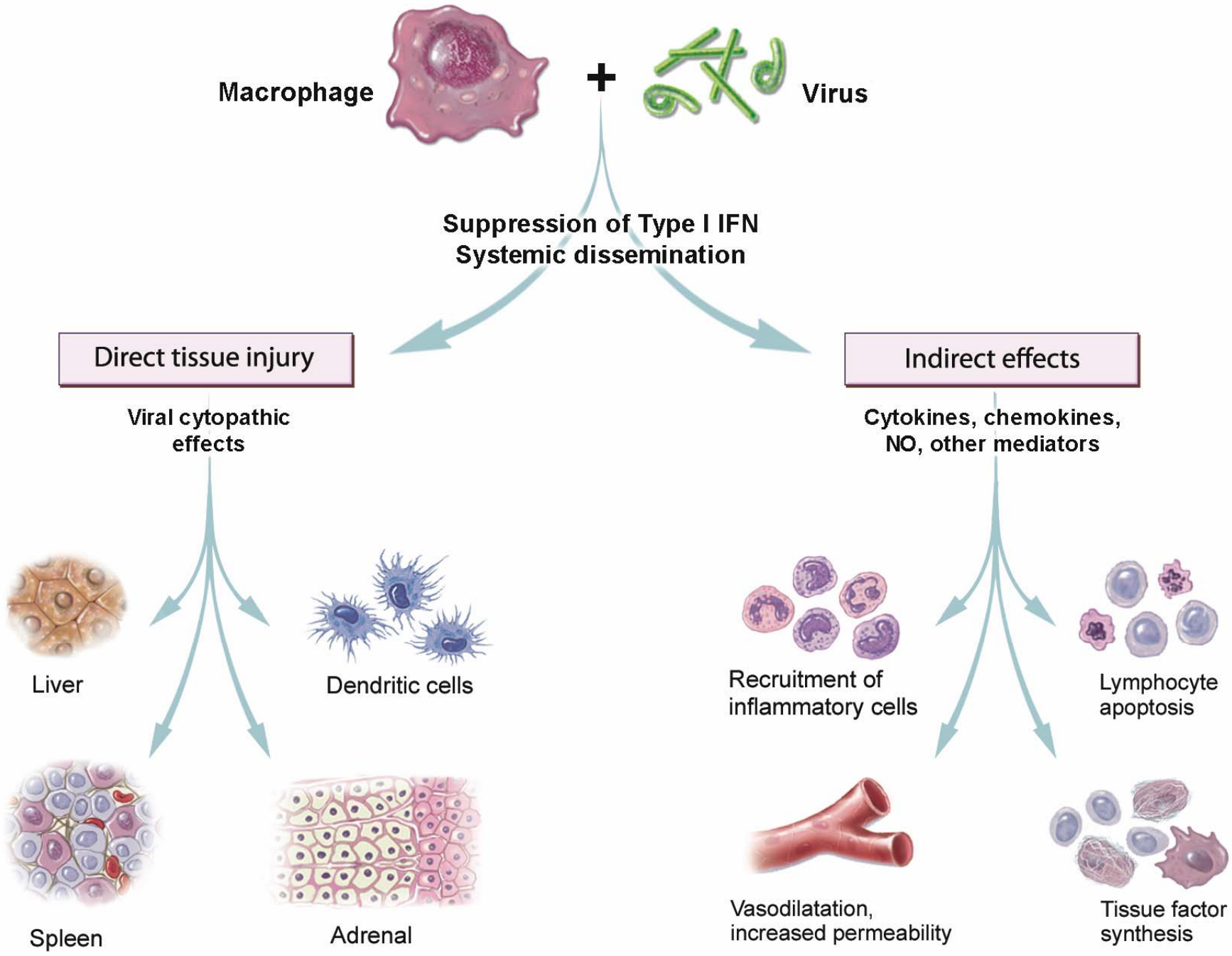
Features of disease in GPs and mice: inflammatory responses

1. TNF-α, IL-6, MCP-1 and other pro-inflammatory cytokines are elevated in the plasma of mice infected with mouse-adapted Ebola virus.
2. Lack of reagents limits testing in guinea pigs.

Why is filoviral HF lethal?

Underlying cause of death is similar in rodents and primates:

1. Rapid spread of virus to macrophages in all tissues produces a systemic inflammatory syndrome.
2. Destruction of dendritic cells and apoptotic loss of lymphocytes prevents an effective adaptive response.
3. Extensive tissue necrosis contributes to disease severity.
4. Hemorrhagic phenomena are common in primates, but rarely the cause of death.


Macrophage
+
Virus
Suppression of Type I IFN
Systemic dissemination
Direct tissue injury
Indirect effects
Viral cytopathic effects
Cytokines, chemokines, NO, other mediators
Liver
Dendritic cells
Recruitment of inflammatory cells
Lymphocyte apoptosis
Spleen
Adrenal
Vasodilatation, increased permeability
Tissue factor synthesis

A "good" animal model

A good animal model of filoviral hemorrhagic fever is one in which inoculation of a small dose of virus leads to:

- rapid systemic spread with high viremia
- infection and necrosis of macrophages and dendritic cells
- release of proinflammatory mediators, leading to increased vascular permeability and shock
- massive lymphocyte apoptosis.

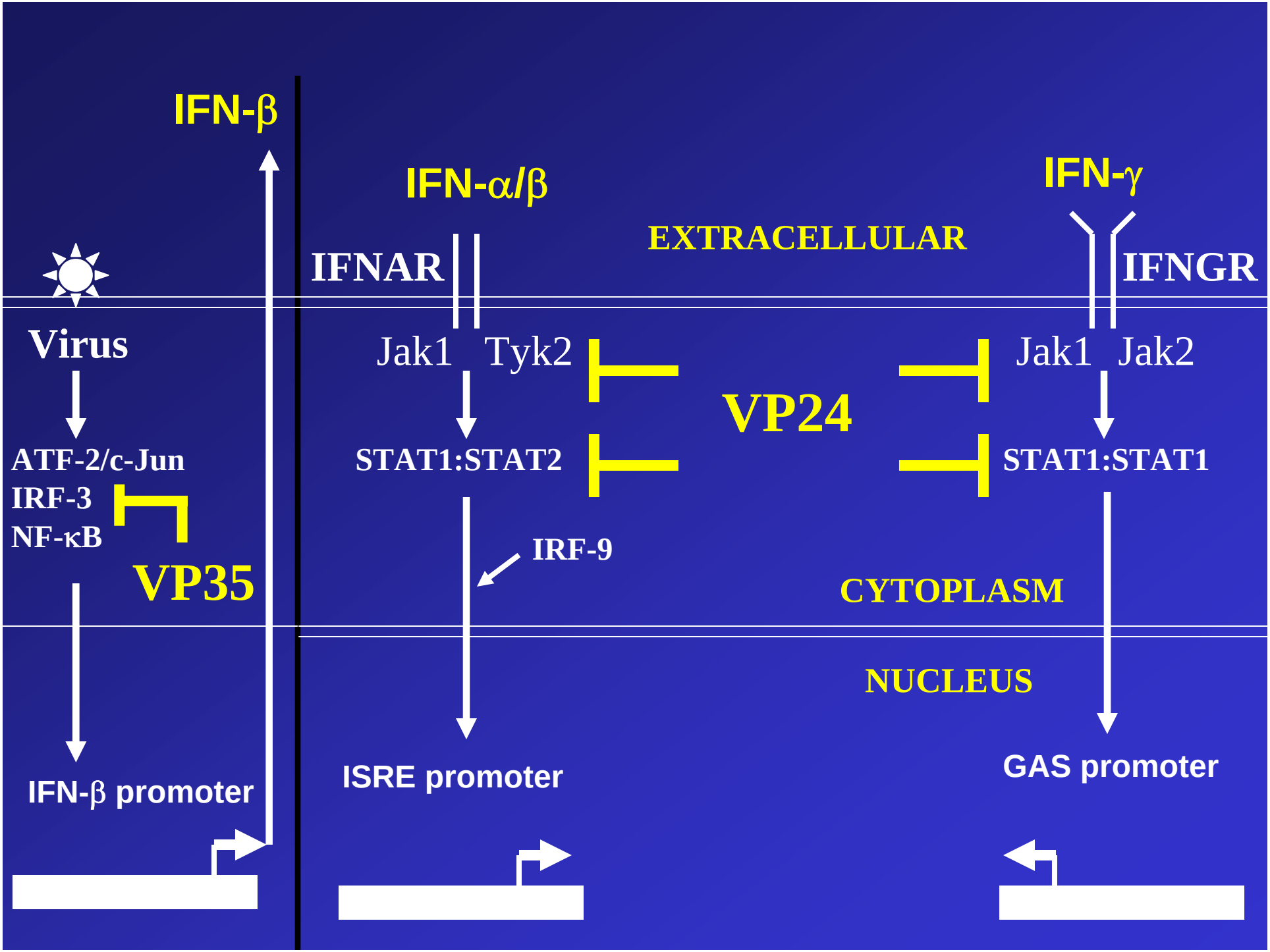
These changes are seen in guinea pigs and mice.

Role of type I IFN in resistance

- Type I IFN responses are much more effective in mice than in primates.
- Treatment with anti-IFN-α antibodies renders normal mice susceptible to lethal infection with wild-type Ebola Zaire virus.
- Mice can be protected against mouse-adapted Ebola virus with IFN or with drugs that induce IFN.

Drug and vaccine testing

- It is typically much easier to protect mice and guinea pigs against filoviral infection than to protect nonhuman primates.
- For example, several types of polyvalent and monoclonal antibodies have protected rodents, but none has yet prevented the death of a primate.
- Many vaccines have also succeeded in rodents, but failed in subsequent nonhuman primate testing.


IFN-β
IFN-α/β
IFN-γ
EXTRACELLULAR
IFNAR
IFNGR
Virus
Jak1
Tyk2
Jak1
Jak2
VP24
ATF-2/c-Jun
IRF-3
NF-κB
STAT1:STAT2
STAT1:STAT1
VP35
IRF-9
CYTOPLASM
NUCLEUS
IFN-β promoter
ISRE promoter
GAS promoter

A better model?

- Would mice deficient in type I IFN responses be a better model for drug and vaccine testing than normal mice?
- STAT-1 KO mice can be lethally infected by wild-type Marburg virus and by Ebola Zaire and Sudan virus.
- This question could be explored by assessing the efficacy of vaccines that have succeeded or failed in nonhuman primates.